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[54] **PROCESS FOR THE PRESERVATION OF PLANT EMBRYOS**

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[21] Appl. No.: **543,475**

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[30] **Foreign Application Priority Data**

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[51] **Int. Cl.**[5] **A01C 1/06**; C12N 5/02; C12N 5/04

[52] **U.S. Cl.** **47/57.6**; 435/240.4; 435/240.49; 47/58

[58] **Field of Search** 435/240.4, 240.49; 47/57.6

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Attorney, Agent, or Firm—Vogt & O'Donnell

[57] **ABSTRACT**

Plant embryos are preserved by coating a plant embryo with oil in an amount sufficient for causing hypoxia and then cooling and storing the coated embryo at a temperature above the cold sensitivity threshold of the embryo.

19 Claims, No Drawings

PROCESS FOR THE PRESERVATION OF PLANT EMBRYOS

BACKGROUND OF THE INVENTION

This invention relates to a process for the preservation of plant embryos.

Numerous species of plants may be preserved and stored in the form of cell suspensions, calluses or even meristems.

The storage of plant embryos is justified in many cases, for example for regulating the production of plantlets where it is seasonal or for maintaining a clonal line. The preservation of plant embryos can have various advantages, including for example the possibility of temporarily stopping the development of the embryos, the time required for their transport to the seed bed or for their storage and the possibility of producing artificial seeds.

Somatic embryos have certain advantages for the multiplication of plants. They emanate in principle from a single cell and give genetically identical plants. From the beginning of their formation, somatic embryos have a bipolar structure: they have the two, meristems i.e., stem and root necessary to produce a plant. Accordingly, somatic embryogenesis appears an interesting alternative for the propagation of plants. It could be used for the rapid multiplication of species that are expensive to produce or of high-performance individuals emanating from in vitro cultures or of transformed plants that are difficult to reproduce sexually for example.

There are various known processes for storing undifferentiated tissues at low temperatures and/or under hypoxia. Generally speaking, the tissues are kept on a semi-solid medium or are stored at a temperature below 0° C.

One known process comprises storing grape calluses at a temperature of 10° C. or 15° C. To increase their storage life, a layer of silicone oil may be added to the calluses, although they remain on their nutrient substrate. Another process which may be applied to yeasts or cells comprises forming an aqueous emulsion of the cells using an oil medium and cooling the whole to a temperature of the order of −20° C.−30° C. so that the water remains supercooled. The oil thus acts as a cryoprotective agent, for the cells or yeasts.

SUMMARY OF THE INVENTION

The object of the present invention is to provide a process for the preservation of plant embryos by which the growth of the embryos isolated from their culture medium may be slowed down significantly for a predetermined time and then resumed without the appearance of secondary embryogenesis.

To this end, the process according to the present invention is characterized in that the embryos are kept under hypoxia by coating with a layer of oil, after which the embryos are cooled and stored at a temperature slightly above the cold sensitivity threshold of the embryos in question.

It has surprisingly been found that, by keeping the embryos under hypoxia in oil at a low temperature, their growth may be partly and durably inhibited without any effect on their viability and germinating power while, at the same time, the integrity of the structure of the embryo during its subsequent growth is maintained without the appearance of any other form of morphogenesis, such as callogenesis or secondary embryogenesis, either in light or in darkness. In other words, the process according to the invention for the preservation of plant embryos on the one hand enables the embryos to survive in their morphological integrity and, on the other hand, enables them effectively to retain their capacity to develop a plantlet.

Accordingly, another advantage of this process is that it enables embryos to be stored for relatively long periods. Another advantage of the process is that it enables embryos to be stored at readily accessible temperatures, for example, in a refrigerator, without having to use expensive equipment. Another advantage of the invention is that it provides a process which can be carried out quickly and easily both in darkness and in light.

DETAILED DESCRIPTION OF THE INVENTION

The embryos used in the present invention may be of any origin and any species, such as carrots or coffee trees, for example.

The embryos may be somatic embryos or zygotic embryos.

The somatic embryos may be obtained from undifferentiated cell suspensions. In this case, seeds of a hybrid parent for example may be aseptically germinated. The hypocotyls may be cut and then placed on a culture medium containing growth hormones. The calluses obtained may then be dissociated in a liquid culture medium. This gives an undifferentiated cell suspension of which the cells, after several subcultures, may be transferred to a culture medium. After about ten days, the cell suspension may be filtered so that only cell aggregates of the required size are retained. These aggregates may be cultured for a few days on a hormone-free culture medium to induce formation of the embryos.

The zygotic embryos may be obtained by sampling by dissection of the seeds at the mature or slightly immature stage.

The somatic and zygotic embryos obtained may be classified according to their stage of development. Preferred embryos are in the initial stages of their development when they are between 150 and 1000 μm in size. These sizes correspond to the heart or torpedo stages of their development.

The embryos obtained may then be washed, for example with a liquid culture medium free from growth hormones of the type typically encountered in embryo culture, such as a Murashige and Skoog medium containing 5 g/l sucrose. The washed embryos may then be transferred to culture plates and dried by withdrawal of the residual culture medium, for example by means of a pipette.

The embryos ar then kept under hypoxia by coating with a layer of oil. The oil is selected for its ability to cause hypoxia, i.e., to transfer little, if any, external oxygen to the embryo. The oil acts as a preservative and provides the embryo with the minimum quantity of oxygen required for its survival. The oil used for maintaining hypoxia may be a mineral oil, an oil of vegetable origin, a synthetic oil or any other oil capable of maintaining hypoxia without being toxic towards the embryos. The oil used is preferably a liquid paraffin oil. The oil may be degassed and/or sterilized beforehand, for example by autoclaving for 20 minutes at 115° C.

The quantity of oil added should be sufficient to coat the embryos completely. The oil is preferably added in a quantity of 0.02 to 0.5 ml per embryo.

The embryos are then cooled to a temperature just above the cold sensitivity threshold of the embryos in question. The cold sensitivity threshold is understood to be the temperature below which the embryos are no longer viable. The temperature to which the embryos are cooled and then stored may be a few degrees, preferably 1° to 10° C., above the cold sensitivity threshold.

The storage temperature may be, for example, between 2° and 8° C. and preferably between 3° and 5° C. for embryos of species that are only slightly sensitive to cold, such as carrots. It may be between 12° and 20° C. and preferably between 15° and 17° C. for embryos of species that are more sensitive to cold, for example species of tropical origin, such as coffee trees for example.

The embryos may be cooled, for example, by transferring the culture plates containing the embryos to a refrigerator or air-conditioned chamber. The cooling rate may be fairly rapid, for example of the order of 1° to 3° C. per minute.

The embryos under hypoxia in oil may be stored in weak light (of the order of 200 lux) or in darkness.

The process according to the invention ensures the survival of embryos stored in darkness. This can have a practical advantage in cases where artificial seeds are subsequently produced by encapsulation of the embryo. This is because it appears probable that no residual lighting reaches the embryo inside the capsule. The embryos may be stored under these conditions for relatively long periods, i.e., for approximately two to four months.

After storage, the embryos may be removed from the refrigerator and reheated to ambient temperature. When they have reached a temperature of the order of 20° C., they may be washed with a typical liquid culture medium to eliminate any trace of oil.

They may then be placed in or on a typical culture medium, such as a Murashige and Skoog medium, where they resume normal growth comparable with that of embryos which have not been stored.

For distribution among users with a view to conventional sowing in a seed bed or in a field, the embryos may be encapsulated in more resistant materials which afford them protection comparable with that of natural seeds. In this case, the oil-coated embryos may be encapsulated in natural or artificial polymers, for example a sodium alginate gel. These capsules afford the embryo mechanical and hygienic protection and provide for feeding of the plantlet during its germination.

The capsules may be subsequently coated with an additional film, for example of a water-soluble resin, which partly protects them against breaking and drying out. It is possible in this way to obtain an artificial seed which keeps for longer periods, namely for the time required for their practical application.

EXAMPLES

The present invention is illustrated in more detail in the following Examples. These Examples are preceded by an example of the conventional preparation of somatic embryos, by the description of a viabjlity test and by Table 1 which gives the composition of the preferred culture medium used.

Example of the Preparation of Somatic Embryos

An undifferentiated cell culture of carrot cells (*Daucus carota L.*) is subcultured every 12 days (1 gram biomass to 100 ml medium) in a Murashige and Skoog liquid culture medium containing 20 g/l sucrose and 0.1 mg/l 2,4-dichlorophenoxyacetic acid.

All handling is carried out under aseptic conditions beneath a laminar flow hood. The suspension is placed on a stirrer making an eccentric gyratory movement of 100 r.p.m. and is cultured at 23° C. under 200 lux lighting with a photoperiod of 16 hours.

After culture for 8 to 10 days, the cell suspension is filtered so that only cell aggregates between 50 and 200 μm in size are retained. These small aggregates represent a proembryonic stage of the embryos which will continue their development to the heart, torpedo and plantlet stages. The aggregates are washed and placed in a Murashige and Skoog medium containing no 2,4-dichlorophenoxyacetic acid in a quantity of approximately 1.5×10^3 aggregates per ml medium.

After culture for 10 days, embryos have formed. The suspension is filtered so that only embryos between 150 and 1000 μm in size are retained.

Viability Test

A quick and simple viability test has been developed to evaluate the viability rate of the embryos after freezing.

Among the various criteria which may be used to evaluate the viability rate of the embryos,

the increase in the size of the embryos and

the appearance of a chlorophyllian coloration are particularly appropriate.

These criteria may be evaluated in various ways, for example by visual counting or by biochemical tests (coloration test for example).

Under the principle of this test, the embryos are placed in a typical liquid or semi-solid culture medium. After culture for 10 days, the number of embryos which have increased in size and show signs of chlorophyllian coloration is recorded. The ratio between this number and the total number of embryos present enables the viability rate of the embryos to be determined.

The embryos may then be kept on the same liquid medium or may be placed on a solid culture medium having the same composition as the preceding liquid medium so that they may continue their development to the plantlet stage. After culture for 10 days on this medium, the conversion level is determined as the ratio between the number of embryos which have developed to the plantlet stage and the total number of embryos.

TABLE 1

Composition of the Murashige and Skoog medium (pH 5.8–6)

Macroelements	mg l^{-1}
NH_4NO_3	1650
$CaCl_2.2H_2O$	440
$MgSO_4.7H_2O$	370
KNO_3	1900
KH_2PO_4	170
Microelements	
$CoCl_2$	0.025
$CuSO_4.5H_2O$	0.025
$FeSO_4.7H_2O$	27.8
Na_2-EDTA	37.3
$MnSO_4.4H_2O$	22.3
KI	0.83
Na_2MoO_4	0.25
$ZnSO_4.7H_2O$	10.6

TABLE 1-continued

Composition of the Murashige and Skoog medium (pH 5.8–6)	
H_3BO_3	6.2
Other elements	
Nicotinic acid	5
Thiamine (vit. B_1)	2
Adenine	2
Sucrose	5,000

EXAMPLE 1

Somatic carrot embryos at the torpedo stage (average size 670 μm) obtained as described above are washed with a liquid Murashige and Skoog culture medium. The embryos are then placed on culture plates consisting of 6 cups in a quantity of 20/30 embryos per cup and are freed from traces of residual culture medium by withdrawal of the liquid with a pipette.

Two groups (A) and (B) of embryos are coated with a layer of liquid paraffin oil sterilized beforehand by autoclaving for 20 minutes at 115° C. 1 ml oil is then added for 5 to 8 embryos. Two control groups (C) and (D) of embryos are coated with the liquid culture medium.

The culture plates are then covered with their cover and hermetically sealed. A first group (A) of embryos under hypoxia in oil is placed in a chamber cooled to 4° C. For comparison, a second group (B) of embryos under hypoxia in oil is kept at 23° C. A first control group (C) of embryos which are not under hypoxia is stored at 4° C. while a second control group (D) is stored at 23° C.

The various groups are stored under light of 200 lux.

After a certain storage time, the embryos are reheated to ambient temperature and a liquid culture medium is injected beneath the layer of oil surrounding the embryos under hypoxia in order to transfer as many embryos as possible to the culture medium.

After elimination of the layer of oil, the embyros are washed in several successive baths of culture medium to eliminate every trace of residual oil.

The development of the size of the embryos during the storage period is observed by measurement of their size with a binocular magnifying glass equipped with a measuring eyepiece.

The following results are obtained: Size of the embryos (μm)

	Storage time (days)					
	0	14	35	49	63	105
Group A (4° C.)	670	674	732	762	854	1327
Group B (23° C.)	670	2863	2896	—	—	—
Control (4° C.)	670	688	1104	1292	1536	3310
Control (23° C.)	670	10500	—	—	—	—

It can be seen that the embryos stored at 23° C. grow fairly rapidly whether or not they are under hypoxia. After 14 days, they have more than tripled in size whereas the embryos stored at 4° C. have hardly grown. After 105 days, the embryos stored at 4° C. under hypoxia in oil are still relatively small in size whereas the control embryos stored at 4° C. in culture medium have continued to grow.

At the same time, it can be seen that storage under hypoxia in oil at 4° C. influences the viability of the embryos.

To this end, the embryos freed from the layer of oil and reheated to ambient temperature are cultured in the Murashige and Skoog liquid culture medium.

The viability of the embryos is evaluated by the resumption or continuation of their growth in liquid medium.

After culture for 10 days, the viability level of the embryos is determined.

The following results are obtained: Viability level: (expressed in % of growth resumption)

	Storage time (days)					
	0	14	35	49	63	105
Group A (4° C.)	97	94	97	92	90	65
Group B (23° C.)	97	96	18	0	—	—
Control (4° C.)	97	92	92	91	87	83
Control (23° C.)	97	90	—	—	—	—

The embryos stored under hypoxia in oil at 4° C. have a correct viability level after storage for 105 days whereas the embryos stored under hypoxia at 23° C. are no longer viable after a storage time of 35 days.

The control embryos stored at 4° C. also show good viability after 105 days, but are then too large in size (approx. 3300 μm) to allow possible storage by encapsulation in a polymer.

After culture for 20 days in the liquid medium, the number of embryos which have developed a normal plantlet is determined.

The following results are obtained: Conversion level (expressed in % of plantlets grown)

	Storage time (days)			
	0	35	63	105
Group A (4° C.)	97	99	80	36
Control (4° C.)	97	86	23	0

The embryos stored at 4° C. under hypoxia in oil continue to grow without the appearance of adventitious proliferation in exactly the same way as the control embryos.

The partial inhibition of the growth of the somatic embryos by keeping them under hypoxia in oil at 4° C. does not affect the viability of the embryos or their ability to resume growth.

EXAMPLE 2

Somatic carrot embryos (average size 460 μm) are stored at 4° C. by the method described in Example 1. The embryos are partly stored in darkness and, for comparison, partly in light of 200 lux.

The development of the size of the embryos during the storage period is observed.

The following results are obtained: Size of the embryos (μm)

	Storage time (days)						
	0	18	29	46	61	81	95
Hypoxia in oil darkness	460	596	601	701	625	545	554
Hypoxia in oil 200 lux	460	610	603	659	547	651	548
Control, darkness	460	999	1325	2811	3644	7454	11670
Control, 200 lux	460	1120	1508	2493	4634	7602	9941

It can be seen that the embryos kept under hypoxia in oil have hardly developed in size whether stored in light or in darkness.

Exposure to light during storage of the embryo does not appear to affect its viability. The absence of light has no effect on the degree of inhibition induced by the combination of hypoxia in oil and a low temperature.

EXAMPLE 3

Somatic embryos of the coffee tree, Coffea arabica, at the advanced torpedo stage (average size 1590 μm) are washed and placed on culture plates by the method described in Example 1.

Three groups (A), (B) and (C) of embryos are coated with a layer of sterilized liquid paraffin oil in a quantity of approximately 1 ml oil for 5 to 8 embryos. Three control groups of embryos are coated with a Murashige and Skoog liquid medium.

One group (A) of embryos under hypoxia in oil is stored at 4° C., a second group (B) at 10° C. and a third group (C) at 15° C. A first control group is stored at 4° C., a second at 10° C. and a third at 15° C.

The groups are kept in darkness for one month and are then returned to ambient temperature. The embryos are washed by the method described in Example 1 and are then cultured on a Murashige and Skoog semi-solid medium.

After culture for 10 days, the viability level of the embryos is determined.

The following results are obtained: Viability level (% growth resumption)

Group A (4° C.)	0	comparison
Group B (10° C.)	0	
Group C (15° C.)	80	
Control (4° C.)	0	comparison
Control (10° C.)	0	
Control (15° C.)	84	

It can be seen that the embryos stored at 4° C. or 10° C. turn brown and die whether they are under hypoxia in oil or in a liquid culture medium.

The coffee tree embryos keep well at a temperature of 15° C.

It is thus important to take into account the cold sensitivity threshold of the species in question for the purpose of determining the minimum storage temperature.

EXAMPLE 4

Somatic coffee tree embryos at the advanced torpedo stage (average size 1590 μm) are stored at 15° C. under hypoxia in oil by the method described in Example 1.

After storage for 1 or 2 months, the embryos are reheated to ambient temperature and washed.

The development of the size of the embryos during the storage period is observed.

The following results are obtained: Size of the embryos (μm)

	Storage time		
	0	1 month	2 months
Embryos under hypoxia in oil	1590	1680	1710
Control embryos	1590	2780	3590

It can be seen that the embryos under hypoxia in oil grow much more slowly than the control embryos.

The viability of the embryos is determined after culture for 10 days on semi-solid medium. It is 70% for the embryos stored for 2 months under hypoxia in oil and 84% for the control embryos stored for 2 months in the liquid culture medium. Coating of the embryos with a layer of oil thus enables their growth to be effectively inhibited without seriously affecting their viability.

EXAMPLE 5

Somatic coffee tree embryos at the advanced heart stage (average size 1100 μm) or at the torpedo stage (average size 1320 μm) are stored in darkness at 15° C. for one month either under hypoxia in paraffin oil or in a liquid culture medium by the method described in Example 1.

After storage, the embryos are reheated to ambient temperature and washed. The evolution of the size of the embryos during the storage period is observed.

The following results are obtained: Size of the embryos (μm)

	Storage time	
	0	1 month
Advanced heart embryos		
Hypoxia in oil	1100	1300
Control	1100	2470
Torpedo embryos		
Hypoxia in oil	1320	1750
Control	1320	2790

As in Example 4, it can be seen that the embryos under hypoxia in oil grow more slowly than the control embryos.

The embryos are cultured for 10 days on a Murashige and Skoog semi-solid medium, after which the viability level is determined.

The following results are obtained:

	Viability level (%)
Advanced heart embryos	
Hypoxia in oil	76
Control	84
Torpedo embryos	
Hypoxia in oil	71
Control	95

We claim:

1. A process for preserving plant embryos comprising coating a plant embryo isolated from culture medium with an amount of oil sufficient for causing hypoxia, cooling the oil-coated embryo to a temperature above a cold sensitivity threshold of the embryo, and storing the oil-coated embryo at a temperature above the cold sensitivity threshold of the embryo.

2. A process according to claim 1 wherein the oil-coated embryo is stored at a temperature which is 1° C. to 10° C. above the cold sensitivity threshold of the embryo.

3. A process according to claim 1 wherein the embryo is a somatic embryo.

4. A process according to claim 1 wherein the embryo is a zygotic embryo.

5. A process according to claim 1 wherein the embryo is a somatic carrot embryo, which is stored at a temperature of 2° C. to 8° C.

6. A process according to claim 1 wherein the embryo is a somatic coffee tree embryo, which is stored at a temperature of 12° C. to 20° C.

7. A process according to claim 1 wherein the oil is selected from the group of oils consisting of mineral oil, oil of vegetable origin, and synthetic oil.

8. A process according to claim 1 wherein the oil is a liquid paraffin oil.

9. A process according to claim 1 wherein the embryo is stored in darkness.

10. A process according to claim 1 wherein the embryo is obtained from an embryo culture, and further comprising, before coating the embryo with oil, washing the embryo with a liquid culture medium and withdrawing residual culture medium from the embryo.

11. A process according to claim 10 wherein the liquid culture medium is free of growth hormones.

12. A process according to claim 1 further comprising encapsulating the oil-coated embryo with a polymer.

13. A process according to claim 12 further comprising coating the polymer-encapsulated oil-coated embryo with a water-soluble resin.

14. An oil-coated plant embryo composition obtained by coating a plant embryo isolated from culture medium with an amount of oil sufficient for causing hypoxia, cooling the oil-coated embryo to a temperature above a cold sensitivity threshold of the embryo, and storing the oil-coated embryo at a temperature above the cold sensitivity threshold of the embryo.

15. An oil-coated plant embryo composition obtained by coating a plant embryo isolated from culture medium with an amount of oil sufficient for causing hypoxia, cooling the oil-coated embryo to a temperature above a cold sensitivity threshold of the embryo, storing the oil-coated embryo at a temperature above the cold sensitivity threshold of the embryo, and encapsulating the oil-coated embryo with a polymer.

16. An oil-coated plant embryo composition obtained by coating a plant embryo isolated from culture medium with an amount of oil sufficient for causing hypoxia, cooling the oil-coated embryo to a temperature above a cold sensitivity threshold of the embryo, storing the oil-coated embryo at a temperature above the cold sensitivity threshold of the embryo, encapsulating the oil-coated embryo with a polymer, and coating the polymer-encapsulated oil-coated embryo with a water-soluble resin.

17. A plant embryo coated with an amount of oil sufficient to cause hypoxia.

18. An oil-coated plant embryo according to claim 17 encapsulated by a polymer.

19. A polymer-encapsulated oil-coated plant embryo according to claim 18 further coated with a water-soluble resin.

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UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,138,793

DATED : August 18, 1992

INVENTOR(S) : FLORIN, et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the title page, under the heading "OTHER PUBLICATIONS", the title of the Augereau, et al., publication should be --Long Term Storage of Callus Cultures at Low Temperatures or Under Mineral Oil--.

Column 1, line 24, after "meristems" insert a comma.

Column 1, line 25, after "root" insert a comma.

Column 1, line 30, "in vitro" should be italicized.

Column 1, line 45, after "-20°C." insert --to--.

Signed and Sealed this

Twenty-first Day of September, 1993

Bruce Lehman

Attest:

BRUCE LEHMAN

Attesting Officer

Commissioner of Patents and Trademarks

US006164012A

United States Patent [19]

Lechelt-Kunze et al.

[11] Patent Number: 6,164,012

[45] Date of Patent: Dec. 26, 2000

[54] **BIOLOGICAL MATERIAL EMBEDDED IN HYDROGELS, A PROCESS FOR THE EMBEDDING THEREOF, AND ITS USE AS ARTIFICIAL SEED**

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§ 371 Date: **Apr. 27, 1999**

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Aug. 2, 1996 [DE] Germany 196 31 320

[51] **Int. Cl.**[7] **A01H 4/00**; A01C 1/06

[52] **U.S. Cl.** **47/57.6**

[58] **Field of Search** 47/57.6, 58.1

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Primary Examiner—Michael J. Carone
Assistant Examiner—Jeffrey L. Gellner
Attorney, Agent, or Firm—Joseph C. Gil; N. Denise Brown

[57] **ABSTRACT**

The invention relates to a completely biodegradable hydrogel comprising polyester polyurethane polyurea containing urea groups as well as polysaccharides and/or their derivatives, the hydrogel containing plant material capable of dividing. The invention also relates to a process for the embedding of the biological material and the fabrication and shaping of the hydrogels from aqueous solutions. The hydrogels according to the invention may be used as coating material for embedding biological material capable of dividing, especially plant material, preferably plant cells, protoplasts, plant tissues and plant organs, as well as zygotic or somatic plant embryos, under sterile conditions for the purpose of protecting the material during storage, transport and handling. The hydrogels according to the invention may also contain further additives, for example, plant protection agents or nutrients. The embedded biological plant material according to the invention may be used as artificial seed.

14 Claims, No Drawings

BIOLOGICAL MATERIAL EMBEDDED IN HYDROGELS, A PROCESS FOR THE EMBEDDING THEREOF, AND ITS USE AS ARTIFICIAL SEED

The present invention relates to a completely biodegradable hydrogel comprising polyester polyurethane polyurea as well as polysaccharides and/or their derivatives, and plant material capable of dividing.

The invention also relates to a process for embedding the biological material and for producing and shaping the hydrogels from aqueous solutions, as well as the use of the biological material embedded in hydrogels as artificial seed.

Plants are propagated sexually via seeds and asexually or vegetatively via meristems of the plants. Both types of propagation are of great economic importance. Whereas sowing of natural seeds is largely carried out mechanically, vegetative propagation involves much manual labour and is therefore more time-consuming, labour-intensive and accordingly more expensive than propagation by seeds.

Plant species, strains, cultivars and lines in which a specific genetic constitution is important (eg. clonal propagation of elite plants) are propagated vegetatively. Vegetative propagation is also used for plants that form seeds only after a long vegetation period, that form only a few seeds, or the germination capacity of whose seeds is damaged.

In order to simplify vegetative plant propagation, in addition to the development of automated processes for large-scale cultivation, suitable substances and processes for encapsulating the fragile material, which furthermore act as a seed case, are also desirable.

In the case of some types of plants it is now possible to produce miniaturised and regenerable plants (tissues) capable of dividing in large-scale cultivation processes (eg. WO95/19102, U.S. Pat. No. 5,294,549, U.S. Pat. No. 5,334, 530). Without mechanical protection and/or protection against desiccation, these plant parts can be transported and stored only to a limited extent. It is therefore desirable to encapsulate or cover plant parts as discrete units so that they can be stored and/or transported, adequately dosed, and used just like natural plant seeds.

DE 2 103 873, EP 141 374, EP 107 141, U.S. Pat. No. 4,562,663, WO 8502972, U.S. Pat. No. 4,779,376, WO 9207457 describe the embedding of plant material in hydrogels which have been produced from ionically crosslinkable polysaccharides such as alginate, gelatins, carragheens or locust bean gum.

The aforementioned materials, combinations of materials and processes according to the prior art have not up to now been completely satisfactory since in some cases they neither impart a sufficient mechanical stability to the coated structures, nor do they protect the plant tissue against a too rapid or too extensive loss of water under conditions of use. This is true in particular of the aforementioned polysaccharide derivatives. During desiccation a marked shrinkage of the materials is also observed, which can seriously affect the protective function of the seed case. A further problem specifically involving the hitherto employed coatings based on polysaccharides such as alginic acids or their salts, or further ionic polysaccharide derivatives, is the insufficient degree of rehydration after a period of desiccation. These materials can therefore be preserved only under appropriate atmospheric humidity levels.

Subsequently applied coatings of fats, oils, waxes or water-insoluble polymers in order to retard dehydration and mechanical stabilisation, as are disclosed for example in U.S. Pat. No. 4,562,663, WO 9217422, U.S. Pat. No. 5,190,

797, are also unsuitable if they have to be processed under unphysiologically high temperatures, require the use of organic solvents, or if they adversely affect the oxygen supply of the enclosed biological material.

Besides hydrogels based on polysaccharides, polyurethane (PU) hydrogels have also been described. DE 3312578 and DE 4 217 891 describe the use of polyurethanes to immobilise cells capable of dividing. In this application PU hydrogels serve as carrier material of cells and biocatalysts in aqueous suspensions, although the PU hydrogels described for this purpose are not biodegradable.

The object of the present invention is to provide a form of encapsulation/packaging of biological material capable of dividing for the purposes of protecting said material during storage, transport and handling, which greatly retards desiccation, is dimensionally stable, is reswellable to a sufficient extent after partial desiccation, is biodegradable, and is easy to produce.

The addition of additives such as nutrients or active and protective substances should also be possible.

The requisite material must be able to be handled under sterile conditions and avoid the use of toxic solvents or physiologically unacceptable conditions.

The aforedescribed objects are surprisingly achieved by the use of completely biodegradable polyester polyurethane polyureas in combination with polysaccharides or polysaccharide derivatives, which may be used as dispersions in water or as aqueous solutions.

It has surprisingly been found that polyester polyurethane polyureas are suitable for coating biological materials and can be used in combination with biodegradable polysaccharides or their derivatives for the embedding in accordance with the invention within the context of the aforedescribed object of the invention.

The present invention relates to biodegradable hydrogels containing at least

A) a polyester polyurethane polyurea, as well as

B) polysaccharides and/or polysaccharide derivatives, and

C) biological material, preferably plant material capable of dividing, especially plant cells, callus tissues, protoplasts, plant tissues or plant organs, for example adventitious shoots, micronodules, axillary buds, apical buds, scions, as well as zygotic or somatic embryos or protocorm analogues.

The plant material may be derived from the following plants: Plants providing nutritional and raw materials, for example cereals (rice, maize, wheat, barley, rye, millet), potatoes, legumes (e.g. lucerne and soybeans), rapeseed, sunflowers, oil palms, sugar cane, sugar beet, sisal, cotton, miscanthus and tobacco; vegetables and root plants (e.g. tomatoes, varieties of cabbage, lettuce, carrots, aubergines, melons, gherkins, asparagus, onions, parsley, ginger); medicinal plants such as ginseng, belladonna, digitalis; fruit (e.g. apples, pears, cherries, grapes, strawberries, citrus fruits, mango, papaya, bananas, nuts); tea, cocoa, coffee bushes; forest trees, for example conifers such as spruce, fir, pine, larch; foliage trees, for example poplar, beech, oak; ornamental plants, for example roses, chrysanthemums, lillies, amaryllis, orchids, geraniums, begonias, pinks, anthurium.

Furthermore, there may preferably be used such biological materials capable of division, which are particularly preferably derived from transgenic plants, in which propagation through seeds or through vegetative organs is no longer possible or possible only with difficulty on account of the nature of the gene technology alteration, e.g. through seed-specific or nodule specific expression of the products.

Hereinafter the term "embedding" describes all possible processes of encapsulation, covering, coating, packaging, etc. of the biological material according to the invention.

The biodegradability of materials is oriented to the requirements under standard conditions (see Example 6).

According to the invention the polyester polyurethane polyureas may be used mixed with ionic or neutral biodegradable polysaccharides and their derivatives in a one-stage or multistage process, in order to form shaped bodies, e.g. spheres, fibres, sheets, coatings or the like.

A water-containing matrix (hydrogel) is formed by the polysaccharides, and the mechanical properties of the hydrogel are surprisingly improved to such an extent by the polyester polyurethane polyurea as to permit the production of simple shaped bodies, for example spheres, and the water loss of the hydrogel as well as of the biological material according to the invention can be controlled.

The polyester polyurethane polyureas used according to the invention are known from DE 19 517 185, now U.S. Pat. No. 5,961,906.

The aforementioned polyureas are prepared by reacting the following while maintaining an equivalent ratio of isocyanate groups to groups reactive with isocyanate groups, of 1:1 to 2:1

a) a diisocyanate component comprising
   a1) hexamethylene diisocyanate or
   a2) mixtures of hexamethylene diisocyanate with a total of up to 60 wt. %, referred to the mixture, of 1-isocyanato-3,3,5-trimethyl-5-isocyanatomethylcyclohexane and/or 4,4'-diisocyanatodicyclohexylmethane and/or 1-methyl-2,4(6)-diisocyanatocyclohexane with

b) a diol component comprising
   b1) at least one polyester diol having a molecular weight, calculated from the hydroxyl group content, of 500 to 10000, of (i) adipic acid and/or succinic acid and (ii) at least one alkane diol with 2 to 6 carbon atoms, or
   b2) a mixture of such polyester diols having up to 32 wt. %, referred to the total weight of component b), of alkane diols optionally having ether groups and containing 2 to 6 carbon atoms,

c) a diamine component in an amount of 2 to 50 equivalent %, referred to the total amount of the groups, reactive to isocyanate groups, present in the components b) and c), comprising
   c1) diaminosulphonates of the general formula

$$H_2N—(—CH_2—)_n—NH—(—CH_2—)_m—SO_3Me$$

   or
   c2) mixtures of diaminosulphonates c1) with up to 70 wt. %, referred to the total weight of component c), of ethylenediamine,

d) optionally hydrophilic polyether alcohols of the general formula

$$H—X—O—R$$

in an amount of up to 10 wt. %, referred to the total weight of the components b), c) and d), as well as

e) optionally water, which is not included in the calculation of the equivalent ratio of isocyanate groups to groups reactive with isocyanate groups,

wherein in the aforementioned general formulae
m and n denote independently integers from 2 to 6,
Me denotes potassium or sodium,
R denotes a monovalant hydrocarbon radical with 1 to 12 carbon atoms, and
X denotes a polyalkylene oxide chain in the molecular weight range 88 to 4000, whose alkylene oxide units comprise at least 40% ethylene oxide units and the remainder propylene oxide units.

Aqueous dispersions of polyester polyurethane polyureas are thus obtained.

The term "aqueous dispersion" is also intended to include aqueous solutions that may be present if the concentration of hydrophilic centres in the polyurethanes containing urea groups is sufficiently high to ensure solubility in water. Often these dispersions are aqueous systems that contain polyurethane having both dispersed and dissolved urea groups.

In order to prepare the aqueous dispersions, the aforementioned starting materials a), b), c) and optionally d) and/or optionally e) are mixed in the aforementioned quantitative ratios.

The diisocyanate component a) consists preferably exclusively of hexamethylene diisocyanate or of a hexamethylene diisocyanate mixture with a total of up to 60 wt. % of 1-isocyanato-3,3,5-trimethyl-5-isocyanatomethylcyclohexane and/or 4,4-diisocyanatodicyclohexylmethane and/or 1-methyl-2,4(6)-diisocyanato-cyclohexane.

The diol component b) comprises either b1) at least one polyester diol or b2) a mixture of at least one polyester diol b1) with up to 32 wt. %, preferably up to 10 wt. %, of at least one alkane diol optionally containing ether groups and having 2 to 6 carbon atoms.

Suitable polyester diols b1) are those having a molecular weight, calculated from the hydroxyl group content, of 500 to 10000 preferably 1000 to 2500 based on (i) adipic acid and/or succinic acid and (ii) alkane diols optionally containing ether groups and having 2 to 6 carbon atoms, for example ethylene glycol, diethylene glycol, 1,4-butanediol, neopentyl glycol and/or 1,6-hexanediol. Particularly preferred are polyester diols in whose preparation ethylene glycol and/or 1,4-butane diol have exclusively been used as diol.

The optionally ether group-containing alkane diols with 2 to 6 carbon atoms that are optionally used as hydroxyl group-containing chain extension agents are those of the type just mentioned above by way of example.

The diamine component c) comprises either c1) diaminosulphonates of the aforementioned general formulae or c2) mixtures of such diaminosulphonates with ethylenediamine, which if used at all are employed in amounts of up to 90 equivalent %, preferably up to 70 equivalent %, referred to the amino groups of component c) that can react with isocyanate groups. Particularly preferred diaminosulphonates are the potassium or sodium salts of N-(2-aminoethyl)-2-aminoethanesulphonic acid.

The diamine component c) is generally used in an amount of 1 to 10 wt.%, preferably 2 to 5 wt. %, referred to the weight of the component b).

The structural component d) that is optionally used is a hydrophilic, monohydric polyether alcohol of the general formula

$$H—X—O—R$$

in which

R and X have the aforementioned meanings.

Preferred are polyether alcohols of the type in which

R denotes an aliphatic hydrocarbon radical with 1to 4 carbon atoms, and

X denotes a polyalkylene oxide chain in the molecular weight range from 500 to 4000, in which at least 40%, in particular at least 70% and particularly preferably 100% of the alkylene oxide units present are ethylene oxide units, the remaining alkylene oxide units being propylene oxide units.

The shaping and simultaneous embedding of the biological material is effected by an ionically induced coacervation of the polyester polyurethane polyurea in which the polysaccharide component is enclosed. The embedding process can be carried out in one step, or also in a multi-stage process. In the one-step process the biological material and polyester polyurethane polyurea are mixed together and coacervated by adding them to an aqueous salt solution. This inclusion process is basically determined by the viscosity of the polyester polyurethane polyurea/polysaccharide mixture used in the solvent that is employed.

In a two-stage process hydrogel spheres comprising a polysaccharide may first of all be produced by choosing a suitable polysaccharide, for example alginate. These hydrogel particles can be provided with a mechanically stable coating by immersion in an aqueous solution of the polyester polyurethane polyurea.

All biodegradable polysaccharides or their derivatives may be used individually or as a mixture as polysaccharide component of the hydrogel according to the invention. Suitable polysaccharides are for example native and soluble starches obtained from any suitable source, amyloses, amylopectin, alginic acids, alginates, carrageenan, chitin, chitosan, dextran, glycogen, guar, carob seed flour, laevan, pectin, pullulan, tamarind seed flour, xanthan and hylan, as well as cellulose obtained from any suitable source. Also suitable are cellulose derivatives, for example cellulose ethers, cellulose esters and cellulose carbamates.

Particularly suitable are for example cellulose ethers such as methyl cellulose, ethyl cellulose or benzyl cellulose with average degrees of subsitution of less than or equal to 2.5, hydroxyethyl cellulose, hydroxypropyl cellulose, dihydroxypropyl cellulose, hydroxybutyl cellulose, methylhydroxyethyl cellulose, methylhydroxypropyl cellulose, methylhydroxybutyl cellulose, ethylhydroxypropyl cellulose, ethylhydroxyethyl cellulose, carboxyalkyl cellulose, sulphoalkyl cellulose, cyanoethyl cellulose and their mixed ethers. Particularly preferred are methyl cellulose, hydroxyethyl cellulose or hydroxypropyl cellulose. Also suitable are polysaccharide derivatives, in particular cellulose derivatives with arbitrary mixtures of ether, ester and carbamate substituents.

The polyurethane polysaccharide combinations according to the invention, hereinafter termed "blend", can be sterilised by autoclaving and are fully biodegradable.

These blends also enable further properties to be monitored and adjusted, namely water content and balance, dimensional stability, permeability to oxygen and nutrients, adjustment of physiological conditions, mechanical breakdown, for example by sprouting plants, as well as the incorporation and permeability of nutrients, protective agents, and active constituents.

It must be considered surprising that the blends possess combinations of properties that are of advantage for the intended use, namely the encapsulation of biological material capable of division. Such combinations of properties include:

the blends can be processed in aqueous solvents.

the blends can be processed at physiological temperatures (18°–30° C.).

the blends can be sterilised by autoclaving without losing their properties.

the blends are fully biodegradable and can be composted.

the blends can be used in simple, economic processes for encapsulation.

the blends are non-toxic to plants.

the blends can be processed so as to ensure water and gas exchange.

the blends result in satisfactory germination rates.

The present invention also provides water-containing embedding compositions for biological material that contain a completely biodegradable polyester polyurethane polyurea and at least one completely biodegradable ionic or neutral polysaccharide or polysaccharide derivative.

The embedding composition preferably comprises at least 20 wt. % of the aforedescribed polyester polyurethane polyurea and at least 0.1 wt. % of a polysaccharide component, for example starch, a starch derivative, cellulose, a cellulose ether, or arbitrary mixtures thereof.

Water-soluble or at least readily swellable polysaccharide derivatives are preferred, for example starch, starch ethers or cellulose ethers, as well as aqueous 5–50 wt. % dispersions of the polyester polyurethane polyurea. Particularly preferred are soluble starches, alginates, methyl cellulose, hydroxyethyl cellulose, methylhydroxypropyl cellulose, methylhydroxyethyl cellulose and/or hydroxypropyl cellulose.

The invention also provides a process for embedding biological material, in which the said biological material is mixed in the presence of an aqueous dispersion of a polyester polyurethane polyurea with a polysaccharide and/or polysaccharide derivative and this mixture is coacervated by contact with an aqueous salt solution. The polysaccharide component and the added biological material are enclosed by means of this ionically induced coacervation of the polyester polyurethane polyurea, this inclusion process basically also being determined by the viscosity of the employed polyester polyurethane polyurea/polysaccharide mixture in the solvent that is used.

The kinematic viscosity of the solution to be ionically crosslinked is preferably greater than 1.1×10^{6} m^2/sec.

The embedding process can be carried out in one step as well as in a multistage process. In the case of a one-stage process the biological material, polyester polyurethane polyurea and polysaccharide component are mixed together and the mixture is coacervated by addition to an aqueous salt solution. Hydrogel particles are formed, which depending on the process can be produced in the form of spheres, tubing, etc. The hydrogel embedding material comprises a blend of polysaccharide and polyester polyurethane polyurea.

In a two-stage process hydrogel spheres consisting of a polysaccharide can first of all be produced by choosing a suitable polysaccharide, for example alginate. These hydrogel spheres are obtained by adding a mixture of polysaccharide and biological material dropwise to a salt solution. The hydrogel particles also contain sufficient amounts of ions for the coacervation of the polyester polyurethane polyurea. These hydrogel particles may accordingly be provided with a mechanically stable coating by immersion in an aqueous solution of the polyurethane polyurea.

In principle therefore, there are at least 2 possible ways of carrying out the embedding process of the biological material in polysaccharide/polyester polyurethane polyurea hydrogels.

In general this process can be varied by altering the combined mixing of biological material, polysaccharide, polyester polyurethane polyurea and ions, the interaction of the polyester polyurethane polyurea and ions always resulting in the coacervation and thus the embedding and shaping,

with the result that this stage has to be carried out last, although arbitrary mixtures of A and B can be used, where mixture A may comprise polysaccharide, polyester polyurethane polyurea and/or biological material, and mixture B may comprise ions, biological material and polysaccharide.

In a particularly preferred one-stage embodiment of the process the polysaccharide component is swelled or dissolved in an aqueous dispersion of the polyester polyurethane polyurea, the biological material is added, and the resultant mixture is coacervated by adding ions, preferably polyvalant ions, especially Ca^{2+}, Mg^{2+}, or Al^{3+}, in the form of their chlorides in a concentration range from 10–1000 mM, a shaping into spheres, fibres, sheets or other moulded bodies being able to be effected by this procedure. The results are hydrogels comprising a blend of polysaccharide and polyester polyurethane polyurea.

In a further preferred two-stage embodiment of the process the biological material is mixed with ions and polysaccharide in an aqueous solvent, and embedding in a polysaccharide hydrogel, which in turn is enveloped by a polyurethane polyurea coating, is carried out by adding the mixture to a polyurethane polyurea dispersion.

In the embedding process nutrients, protective substances and active agents that promote the growth or metabolism of the biological material to be embedded, and also protect the latter against harmful influences, may be added to the embedding compositions.

In a preferred embodiment the embedding composition may be prepared in a semi-concentrated nutrient medium having the composition specified by Murashige and Skoog (published in Physiol. Plant. 15, 473, 1962), to which 5–20 g/l of sucrose, but preferably 10 g/l of sucrose, have been added.

Any other nutrient salt mixtures that are for example commercially available, as well as sugar, may also be used depending on the embedded plant material. The nutrient media may contain phytohormones known to the person skilled in the art in order to influence the development. Depending on the plant material, the nutrients include the conventional and commercially available nutrient salt mixtures and vitamin mixtures as well as, optionally, likewise commercially available natural or synthetic phytohormones, for example from the class of auxins, cytokinins, gibberelins, abscisic acid, as well as ethylene-forming substances. In addition, compounds that have vitamin-like or phytohormone-like effects, for example chlorocholine chloride, lipo-oligosaccharides, salicylic acid derivatives, may also be used.

In a particular embodiment bactericidal, fungicidal, insecticidal, acaricidal, nematicidal and, in the case of appropriate natural tolerance or tolerance imparted by gene technology, also herbicidal active substances may be added to the embedding material in order to protect the dividing plant material. Protective substances include for example insecticides, for example from the class of phosphoric acid esters, carbamates, especially Imidacloprod, or for example fungicides from the classes of azoles, especially Triadimenol and Tebuconazol.

The following may be mentioned as examples of fungicides:

2-Aminobutane; 2-Anilino-4-methyl-6-cyclopropyl-pyrimidine; 2,6'-Dibromo-2-methyl-4'-trifluoromethoxy-4'-trifluromethyl- 1, 3-thiazol-5-carboxanilide; 2,6-Dichloro-N-(4-trifluoromethylbenzyl)-benzamide; (E)-2-Methoximino-N-methyl-2-(2-phenoxyphenyl)-acetamide; 8-Hydroxyquinoline sulphate; Methyl-(E)-2-{2-[6-(2-cyanophenoxy)-pyrimidin-4-yloxy]-phenyl}-3-methoxy acrylate; Methyl-(E)methoximino [alpha-(o-tolyloxy)-o-tolyl]-acetate; 2-Phenylphenol (OPP),

Aldimorph, Ampropylfos, Anilazin, Azaconazol,

Benalaxyl, Benodanil, Benomyl, Binapracyl, Biphenyl, Bitertanol, Blasticidin-S, Bromuconazole, Bupirimate, Buthiobate,

Calcium polysulphide, Captafol, Captan, Carbendazim, Carboxin, Quinone methionate (Quinomethionate), Chloroneb, Chloropicrin, Chlorothalonil, Chlozolinat, Cufraneb, Cymomanil, Cyproconazole, Cyprofuram,

Dichlorophen, Diclobutrazol, Dichlofluanid, Diclomezin, Dicloran, Diethofencarb, Difenoconazol, Dimethirimol, Dimethomorph, Diniconazol, Dinocap, Diphenylamine, Dipyrithion, Ditalimfos, Dithianon, Dodine, Drazoxolon,

Edifenphos, Epoxyconazole, Ethirimol, Etridiazol,

Fenarimol, Fenbuconazole, Fenfuram, Fenitropan, Fenpiclonil, Fenpropidin, Fenpropimorph, Fentin acetate, Fentin hydroxide, Ferbam, Ferimzone, Fluazinam, Fludioxonil, Fluoromide, Fluquinconazole, Flusilazole, Flusulfamide, Flutolanil, Flutriafol, Folpet, Fosetyl-Aluminium, Fthalide, Fuberidazol, Furalaxyl, Furmecyclox,

Guazatine,

Hexachlorobenzene, Hexaconazol, Hymexazol,

Imazalil, Immibenconazol, Iminoctadin, Iprobenfos (IBP), Iprodion, Isoprothiolan,

Kasugamycin, copper preparations, such as: copper hydroxide, copper naphthenate, copper oxychloride, copper sulphate, copper oxide, Oxin-copper and Bordeaux mixture,

Mancopper, Mancozeb, Maneb, Mepanipyrim, Mepronil, Metalaxyl, Metconazol, Methasulfocarb, Methfuroxam, Metiram, Metsulfovax, Myclobutanil,

Nickel dimethyldithiocarbamate, nitrothal-isopropyl, Nuarimol,

Ofurace, Oxadixyl, Oxamocarb, Oxycarboxin,

Pefurazoate, Penconazol, Pencycuron, Phosdiphen, Pimaricin, Piperaline, Polyoxin, Probenazol, Prochloraz, Procymidon, Propamocarb, Propiconazole, Propineb, Pyrazophos, Pyrifenox, Pyrimethanil, Pyroquilon,

Quintozen (PCNB),

Sulphur and sulphur preparations,

Tebuconazol, Tecloftalam, Tecnazen, Tetraconazol, Thiabendazol, Thicyofen, Thiophanate-methyl, Thiram, Tolclophos-methyl, Tolyl fluanide, Triadimefon, Triadirnenol, Triazoxid, Trichlamid, Tricyclazol, Tridemorph, Triflumizol, Triforin, Triticonazol,

Validamycin A, Vinclozolin,

Zineb, Ziram,

8-tert-Butyl-2-(N-ethyl-N-n-propyl-amino)-methyl-1,4-dioxa-spiro-[4,5]decane, N-(R)-(1-(4-chlorophenyl)-ethyl)-2,2-dichloro-1-ethyl-3t-methyl-1r-cyclopropanecarboxylic acid amide (mixture of diastereomers or individual isomers), [2-methyl-1-[[[1-(4-methylphenyl)-ethyl]-amino]-carbonyl]-propyl]-carbamic acid-1-methylethyl ester and

1 -methyl-cyclohexyl-1-carboxylic acid-(2,3-dichloro4-hydroxy)-anilide.

The following may be mentioned as examples of bactericides:

Bronopol, Dichlorophen, Nitrapyrin, Nickel dimethyldithio carbamate, Kasugamycin, Octhilinon, Furanecarboxylic acid, Oxytetracycline, Probenazol, Streptomycin, Tecloftalam, copper sulphate and other copper preparations.

The following may be mentioned as examples of insecticides, acaricides and nematicides:

Abamectin, Acephate, Acrinathrin, Alanycarb, Aldicarb, Alphamethrin, Amitraz, Avermectin, AZ 60541, Azadirachtin, Azinphos A, Azinphos M, Azocyclotin,

Bacillus thuringiensis, 4-Bromo-2-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile, Bendiocarb, Benfuracarb, Bensultap, Betacyfluthrin, Bifenthrin, BPMC, Brofenprox, Bromophos A, Bufencarb, Buprofezin, Butocarboxin, Butylpyridaben,

Cadusafos, Carbaryl, Carbofuran, Carbophenothion, Carbosulfan, Cartap, Chloethocarb, Chloretoxyfos, Chlorfenvinphos, Chlorfluazuron, Chlormephos, N-[(6-Chloro-3-pyridinyl)-methyl]-N'-cyano-N-methyl-ethanimidamide, Chlorpyrifos, Chlorpyrifos M, cis-Resmethrin, Clocythrin, Clofentezin, Cyanophos, Cycloprothrin, Cyfluthrin, Cyhalothrin, Cyhexatin, Cypermethrin, Cyromazin,

Deltamethrin, Demeton-M, Demeton-S, Demeton-S-methyl, Diafenthiuron, Diazinon, Dichlofenthion, Dichlorvos, Dicliphos, Dicrotophos, Diethion, Diflubenzuron, Dimethoate, Dimethylvinphos, Dioxathion, Disulfoton,

Edifenphos, Emamectin, Esfenvalerate, Ethiofencarb, Ethion, Ethofenprox, Ethoprophos, Etrimphos,

Fenamiphos, Fenazaquin, Fenbutatinoxide, Fenitrothion, Fenobucarb, Fenothiocarb, Fenoxycarb, Fenpropatirin, Fenpyrad, Fenpyroximat, Fenthion, Fenvalerate, Fipronil, Fluazinam, Fluazuron, Flucycloxyron, Flucythrinat, Flufenoxuron, Flufenprox, Fluvalinate, Fonophos, Formothion, Fosthiazat, Fubfenprox, Furathiocarb,

HCH, Heptenophos, Hexaflumuron, Hexythiazox,

Imidacloprid, Iprobenfos, Isazophos, Isofenphos, Isoprocarb, Isoxathion, Ivermectin,

Lambda-cyhalothrin, Lufenuron,

Malathion, Mecarbam, Mevinphos, Mesulfenphos, Metaldehyde, Methacrifos, Methamidophos, Methidathion, Methiocarb, Methomyl, Metolcarb, Milbemectin, Monocrotophos, Moxidectin,

Naled, NC 184, Nitenpyram,

Omethoate, Oxamyl, Oxydemethon M, Oxydeprofos,

Parathion A, Parathion M, Permethrin, Phenthoate, Phorate, Phosalon, Phosmet, Phosphamidon, Phoxim, Pirimicarb, Pirimiphos M, Pirimiphos A, Profenophos, Promecarb, Propaphos, Propoxur, Prothiophos, Prothoate, Pymetrozin, Pyrachlophos, Pyridaphenthion, Pyresmethrin, Pyrethrum, Pyridaben, Pyrimidifen, Pyriproxifen,

Quinalphos,

Salithion, Sebufos, Silafluofen, Sulfotep, Sulprofos,

Tebufenozide, Tebufenpyrad, Tebupirimiphos, Teflubenzuron, Tefluthrin, Temephos, Terbam, Terbufos, Tetrachlorvinphos, Thiafenox, Thiodicarb, Thiofanox, Thiomethon, Thionazin, Thuringiensin, Tralomethrin, Triarathen, Triazophos, Triazuron, Trichlorfon, Triflumuron, Trimethacarb,

Vamidothion, XMC, Xylylcarb, Zetamethrin.

Chemical or biological agents that induce resistance and that protect the plants against phytopathogenic microorganisms such as fungi, bacteria, viruses or viroids, may also be used as protective substances. Many compounds with a resistance-inducing action provide protection against insects or nematodes. Examples of classes of substances having a resistance-inducing action include benzothiadiazole and its derivatives, mono- and dichloroisonicotinic acids and their derivatives, dichloroisothiazole and its derivatives, dibromothiophencarboxylic acids and their derivatives, salicylic acid and its derivatives, as well as Probenazole. Biological resistance-inducing agents include microorganisms, for example fungi, bacteria or viruses useful to the plant, and which provide protection for the plant against pathogenic organisms, for example against harmful fungi, bacteria, viruses or nematodes.

In addition to such microorganisms, there may also be used in the artificial seeds according to the invention organisms that act as symbionts, for example mycorrhizal-fungi, or that promote plant growth, like for example rhizobia in connection with nitrogen fixation. Also, by the formation of specific metabolic products by microorganisms that are used in combination with the plant material, the germination and growth of the plants can be improved and the plants can be protected against pathogens and attack by pests.

The hydrogel embedding compositions according to the invention can be used as a way of storing or transporting biological material.

The invention also provides for the use of the resultant embedded biological materials as artificial seeds.

The biodegradability of the polyester polyurethane polyureas according to the invention as well as the mixtures with the polysaccharide derivatives according to the invention was demonstrated as described hereinafter. The biodegradability of the embedding compositions formed from the materials according to the invention was also demonstrated in compost and soil. The material had completely degraded after at most 4 weeks, a control experiment with a biologically inactive substrate did not show any decomposition, and accordingly a disintegration of the embedding composition by hydrolysis or mechanical influences can be excluded. The degradation also occurred in the presence of the specified additives according to the invention, for example active constituents, nutrients, etc.

The compounds to be tested are buried in a 2 cm-high mixture of completely rotted compost from a composting unit, degree of rotting IV, in a suitable box. The filled boxes are incubated in an incubating cabinet for in each case 4 weeks in succession at 60°, 50° and 37° C. Water losses are determined from the weight loss and are replenished. During incubation the pH of the compost is regularly measured. If the measured value deviates by more than 1 unit from pH 7, the pH is readjusted to 7.0 by adding 100 mM potassium phosphate. At weekly intervals incubation of a batch is discontinued, the materials are removed, purified, dried to constant weight at 80° C., and photographed. Immediately after drying the weight loss of the material was determined by renewed weighing.

In the poisoned control, the incubation batch is completely dried at 105° C. and the evaporated water is then replaced by a 0.1% $HgCl_2$ solution. The samples for the poisoned control are placed in the $HgCl_2$ solution and then dried, before being added to the compost mixture. The control batch is incubated in exactly the same way as the batches to be tested. A substance is classified as biodegradable if, after 4 weeks, sample substances can no longer be

detected in the unpoisoned batch whereas the sample in the poisoned batch remains unchanged.

The invention will now be illustrated in more detail with the aid of the following examples, though without being restricted by the latter.

EXAMPLES

In the examples the polyester polyurethane polyurea according to DE 19 517 185, now U.S. Pat. No. 5,961,906 is used as polyester polyurethane polyurea. Water-soluble, biodegradable hydroxyalkyl cellulose ethers with a mean molecular weight (number average) of ca. 10000 to 200000 g/mole and a degree of substitution as regards the ether groups of ca. 0.5 to 1.5 are used as hydroxyethyl cellulose or hydroxypropyl cellulose in the examples.

Example 1

Potato plants (*Solanum tuberosum*) were propagated in vitro. For this purpose shoot cuttings with 2 to 6 small leaves were placed in liquid BM medium containing 20 g/l of sucrose and incubated in a plant cabinet under a light/dark rhythm of 12 hours each at 22° C. during the day-time and 19° C. during the night-time. The BM medium consisted of salts according to Murashige/Skoog (cf. Murashige T., Skoog, Physiol. Plant. 15, 473–479, 1962) and vitamins corresponding to Gamborg's Medium B5 (Gamborg O. L., Miller R. A., Ojima K., Exp. Cell. Res. 50, 151, 1968, Gamborg O. L., Murashige T., Thorpe T. A., Vasil I. K., In Vitro 12 473, 1976). After 3 to 4 weeks shoot cuttings were taken from these plants and used for encapsulation experiments.

The shoot cuttings were suspended under sterile conditions in a 3% dispersion of hydroxypropyl cellulose (HPC; with addition of 0.2 M $CaCl_2$ in semi-concentrated nutrient solution according to Murashige-Skoog) and added dropwise while stirring to a 1% alginate solution. The spheres were next washed, while stirring, with a 0.2 M $CaCl_2$ solution. The spheres were then added while gently stirring to a 5% aqueous dispersion of polyester polyurethane polyurea, a thin, elastic coating of polyester polyurethane polyurea forming on the surface of the sphere.

After 5 minutes the spheres, which now had a diameter of ca. 5 mm, were removed from the solution and washed with 0.2 M $CaCl_2$ solution. The spheres were then laid out for germination on agar plates containing semi-concentrated Murashige-Skoog nutrient medium. Incubation was carried out at 20° C. under 12 hours' light per day in a plant cabinet.

After ca. 2 to 3 weeks small plants were growing on the polymer spheres. The germination rate was 66%.

Example 2

The biological material to be encapsulated and derived from potato plants (cultivated according to Example 1) was suspended under sterile conditions in a 3% solution of sodium alginate. The suspension was added dropwise to a 0.2 M $CaCl_2$ solution, resulting in the formation of alginate spheres. After 30 minutes the spheres were suctioned off and added to a gently stirred 5% aqueous polyester polyurethane polyurea dispersion. A thin elastic coating of polyester polyurethane polyurea formed on the surface of the alginate hydrogels. After 5 minutes the spheres were removed from the solution and if necessary washed once more in a 0.1 M $CaCl_2$ solution. For germination, the seeds, which had a diameter of ca. 5 mm, were placed on agar plates with semi-concentrated Murashige-Skoog nutrient medium. Incubation was carried out, as described in Example 1, in the plant cabinet.

Example 3

75 ml of a 40% dispersion of a polyester polyurethane polyurea and 75 ml of a 2% dispersion of hydroxyethyl cellulose are each autoclaved individually at a temperature of 121° C. for 20 minutes and then mixed under sterile conditions in a ratio of 1:1.

The potato shoot cuttings were applied under sterile conditions to the surface of this mixture of hydroxyethyl cellulose and polyester polyurethane polyurea and sucked off individually by means of a pipette.

The shoot cuttings together with the surrounding mixture of hydroxyethyl cellulose and polyester polyurethane polyurea are then added dropwise to a 0.2 M $CaCl_2$ solution. After a residence time of 10 minutes the spheres, which have a diameter of ca. 5 mm, were removed and laid out on agar with semi-concentrated MS medium. Incubation was carried out at 20° C. under 12 hours' illumination per day in the plant cabinet. The ger mination rate was 9 0% within 2 to 3 weeks.

Example 4

75 ml of a 40% dispersion of a polyester polyurethane polyurea and 75 ml of a 2% dispersion of hydroxypropyl cellulose were each autoclaved individually at a temperature of 121° C. for 20 minutes and then mixed under sterile conditions in a ratio of 1:1.

The potato plants were propagated in vitro (cf. Example 1). After 3 to 4 weeks shoot cuttings were taken from these plants and used for encapsulation experiments. The shoot cuttings were placed under sterile conditions on the surface of the mixture of hydroxypropyl cellulose and polyester polyurethane polyurea and sucked off individually by means of a pipette.

The shoot cuttings together with the surrounding mixture of hydroxypropyl cellulose and polyester polyurethane polyurea were then added dropwise to a 0.2 M $CaCl_2$ solution. After a residence time of 10 minutes the spheres, which have a diameter of ca. 5 mm, were removed and placed on agar with semi-concentrated MS medium.

Incubation was carried out at 20° C. under 12 hours' illumination per day in the plant cabinet. The germination rate was between 90% and 100% within 2 to 3 weeks.

Example 5

A cell suspension of carrots (*Daucus carota*) was incubated in 50 ml of hormone-containing Murashige-Skoog medium (MS medium, cf. Murashige T., Skoog, F., Physiol. Plant. 15, 473–479, 1962) at 25° C. and 100 revs. per minute on a mechanical shaker in the dark.

After 8 days 150 ml of the cell suspension was screened through a sieve of mesh width 500 μm, 75 μm and 30 μm. The 30 μm to 75 μm cell fraction was rinsed with hormone-free medium, sedimented by centrifugation at 100 g, washed twice with hormone-free MS medium and, after renewed centrifugation, taken up in 20 ml of hormone-free MS medium. The cell count was as a rule 0.5×10^4 to 10^5 cells/ml.

These cells were used to induce embryogenesis. The screened, washed cells were, as described above, incubated further on the mechanical shaker; after 2 days and 5 days there was a change of medium, the cells being centrifuged off and resuspended in hormone-free MS medium. The cells were then incubated for a further 9 days. After a total of 14 days the suspension contained 10 to 100 embryoids/ml.

Somatic carrot embryos of the "torpedo" and "cotelydonary" stages were applied to the surface of a mixture of hydroxypropyl cellulose and polyester polyurethane polyurea. The embryos were sucked off individually using a pipette and added dropwise together with the surrounding polymer mixture to a 0.2 M $CaCl_2$ solution. After a residence time of 10 minutes the spheres, which have a diameter of ca. 5 mm, were removed and placed on agar with semi-concentrated MS medium. The incubation was carried out at 20° C. under 12 hours' illumination per day in the plant cabinet. After 2 weeks 20% of the spheres had germinated.

Example 6

Examination of the biodegradability of the encapsulations.

The encapsulations obtained from Examples 1–5 were, as described hereinbefore, tested in a composting experiment as regards their complete biodegradability. The degradation was checked at intervals of a few days. The control experiment in poisoned compost shows that microbial decomposition takes place.

Example	7 days	20 days	32 days	42 days	Control
1	intact	discoloured	incipient decomposition	decomposed	intact
2	intact	discoloured	incipient decomposition	decomposed	intact
3	intact	discoloured	incipient decomposition	decomposed	intact
4	intact, discoloured	discoloured	incipient decomposition	decomposed	intact
5	intact, discoloured	discoloured	incipient decomposition	decomposed	intact

Example 7

75 ml of a 40% dispersion of a polyester polyurethane polyurea and 75 ml of a 2% dispersion of hydroxypropyl cellulose which additionally contains 2% of Imidacloprid were each autoclaved individually at a temperature of 121° C. for 20 minutes and then mixed under sterile conditions in a ratio of 1:1. This mixture was added dropwise to a 0.2 M $CaCl_2$ solution.

The resulting ca. 5 mm large spheres contain ca. 30 mg/g of the active constituent.

Example 8

Drying/rehydration.

The shperes prepared in Examples 1 to 5 were dried for 7 days under normal atmospheric conditions and then weighed. After 24 hours' storage in water, a weight increase of around 45% was measured, which did not increase any further even after prolonged storage in water.

Example 9

Combination with active components.

75 ml of a 40% dispersion of a polyurethane polyurea and 75 ml of a 2% dispersion of hydroxypropyl cellulose were each autoclaved individually at a temperature of 121° C. for 20 minutes and then mixed under sterile conditions in a ratio of 1:1.

A solution of the herbicide Imidacloprid (1 mole/l in DMF) was sterile filtered through a membrane filter (pore width 0.2 μm) and then diluted 1:10 with sterile water. The resultant stock suspension of Imidacloprid was added to the mixture of polyurethane polyurea and hydroxypropyl cellulose to give an end concentration of 0.1 mM/l. A sterile 0.2 M $CaCl_2$ solution contains Imidacloprid in the same end concentration.

The potato shoot cuttings (cf. Example 1) were placed under sterile conditions on the surface of the mixture of hydroxypropyl cellulose and polyurethane polyurea and sucked off individually by means of a pipette.

The shoot cuttings together with the surrounding mixture of hydroxypropyl cellulose and polyurethane polyurea were then added dropwise to a 0.2 M $CaCl_2$ solution. Potato shoot cuttings without Imidacloprid were encapsulated as a control test. After a residence time of 10 minutes the spheres were removed and placed on agar with semi-concentrated MS medium. Incubation was carried out at 20° C. under 12 hours' illumination per day and 70% atmospheric humidity in the plant cabinet.

The germination rate was 64% within 4 weeks; the control without Imidacloprid showed a germination rate of 57%.

What is claimed is:

1. A hydrogel comprising:

A) at least one polyester polyurethane polyurea,

B) at least one polysaccharide, a derivative thereof, or a mixture thereof, and

C) at least one biological material.

2. The hydrogel of claim 1, wherein C) said biological material comprises biological plant material that is capable of dividing.

3. The hydrogel of claim 2, wherein C) said biological plant material is selected from the group consisting of (1) plant cells, (2) callus tissue, (3) protoplasts, (4) plant tissue, (5) plant organs, (6) zygotic embryos (7) somatic embryos and (8) protocrom analogues.

4. The hydrogel of claim 3, wherein C)(5) the plant organs comprise adventitious shoots, micronodules, auxiliary buds, apical buds and/or scions.

5. The hydrogel of claim 1, wherein C) said biological material is capable of dividing from transgenic plants.

6. The hydrogel of claim 1, wherein A) the polyester polyurethane polyurea comprises the reaction product of a) an organic diisocyanate component, with b) a diol component, c) a diamine component, and optionally, d) a hydrophilic polyether alcohol, optionally, in the presence of e) water, wherein the water is not included in the calculation of the equivalent ratio of isocyanate groups to isocyanate-reactive groups.

7. The hydrogel of claim 6, wherein A) the polyester polyurethane polyurea is prepared from a) an organic diisocyanate component which is selected from the group consisting of a)1) hexamethylene diisocyanate, and a)2) a mixture of hexamethylene diisocyanate with a total of up to 60% by weight, based on 100% by weight of the mixture, of at least one compound selected from the group consisting of (i) 1-isocyanato-3,3,5-trimethyl-5-isocyanatomethyl-cyclohexane, (ii) 4,4'-diisocyanatodicyclohexylmethane, and (iii) 1-methyl-2,4(6)-diisocyanatocyclohexane.

8. The hydrogel of claim 1, wherein B) the polysaccharide and/or derivative thereof comprises at least one compound selected from the group consisting of soluble starch, alginates, methyl cellulose, hydroxyethyl cellulose, methylhydroxypropyl cellulose, methylhydroxyethyl cellulose, hydroxylpropyl cellulose, and mixtures thereof.

9. The hydrogel of claim 1, which additionally comprises one or more of the following components: a suitable nutrient salt mixture for plant breeding, a bactericidal constituent, a fungicidal constituent, an insecticidal constituent, an acaricidal constituent, a nematicidal constituent, a resistance-inducing constituent, and a herbicidal active constituent.

10. An artificial seed comprising the hydrogel of claim 1.

11. An embedding composition for biological materials, wherein the embedding composition comprises A) at least one polyester polyurethane polyurea, and B) at least one polysaccharide, a derivative thereof, or a mixture thereof.

12. The embedding composition of claim 11, which comprises an aqueous dispersion of A) 5 to 50% by weight of at least one polyester polyurethane polyurea, and B) 0.1% by weight or more of at least one polysaccharide, a derivative thereof, or a mixture thereof.

13. A process for the production of a biological material embedded in a hydrogel comprising

1) mixing the biological material in the presence of an aqueous dispersion of a polyester polyurethane polyurea, with a polysaccharide, a derivative thereof or a mixture thereof,

and

2) coacervating the mixture formed in step 1) by contact with a salt solution.

14. The process of claim 13, wherein the salt solution in step 2) comprises a salt solution of polyvalent ions.

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[54] **METHOD FOR STORING GEL-COATED SEEDS**

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[58] **Field of Search** 47/57.6, 58

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[57] **ABSTRACT**

A method for storing gel-coated seeds having a gel coat comprising an aqueous gel having been water-insolubilized by a metal ion, which comprises storing the gel-coated seeds in an aqueous solution containing the metal ion.

5 Claims, No Drawings

METHOD FOR STORING GEL-COATED SEEDS

FIELD OF THE INVENTION

The present invention relates to a method for storing gel-coated seeds of plants.

BACKGROUND OF THE INVENTION

Coating or encapsulating seeds with gel facilitates planting the seeds in the ground and is effective to accelerate germination as described in WO 87/01258. Gel-coated seeds are prepared by coating seeds with gel insolubilized in water by metal ions, e.g., a calcium ion, so as to have a uniform size and an appropriately controlled hardness. The gel coating technique has made it feasible to mechanize planting of even those seeds that are too small to be planted mechanically. Furthermore, because the gel coat surely supplies to the encapsulated seed water necessary for germination, the gel-coated seeds achieve an improved rate of germination.

Preparation of gel-coated seeds requires equipment and chemicals, it is demanded for working efficiency to prepare a large quantity of gel-coated seeds at a time and store them so that they may be planted later according to a planting schedule. However, if gel-coated seeds are stored under the same conditions as for general uncoated seeds, the water content of the gel coat would be reduced, and it is difficult for the gel coat to maintain a water content necessary for germination. Furthermore, the gel coat would be hardened due to loss of water, making it difficult for a bud or a root sprouted from the seed to pierce through the coat and stick out, which results in reduction in yield.

Once a gel coat of a gel-coated seed loses its water content, it is difficult to let the gel coat re-absorb water to restore its original shape and properties. That is, the surface of the gel coat tends to be peeled, the handling properties of the gel-coated seeds are seriously deteriorated, and the gel strength of the gel coat is extremely reduced, resulting in a failure of mechanical planting.

In order to solve the above problem, it has been proposed to incorporate a water-absorbing polymer into the gel coat as disclosed in JP-A-5-56707 (the term "JP-A" as used herein means an "unexamined published Japanese patent application"). However, addition of a water-absorbing polymer not only incurs an increase in viscosity, which makes gel coat formation difficult, but reduces clarity of the gel coat and thereby impairs visibility of the encapsulated seed, which makes it difficult to control the conditions of germination acceleration which is to be conducted before planting.

SUMMARY OF THE INVENTION

In the light of the above-mentioned disadvantages of conventional techniques, an object of the invention is to provide a method for storing gel-coated seeds easily without causing reductions in yield and handling properties.

This and other objects of the present invention have been accomplished by a method for storing gel-coated seeds having a gel coat comprising an aqueous gel having been water-insolubilized by a metal ion, which comprises storing the gel-coated seeds in an aqueous solution containing said metal ion (hereinafter referred to as a storage solution).

DETAILED DESCRIPTION OF THE INVENTION

Gel-coated seeds having a gel coat comprising an aqueous gel having been water-insolubilized by a metal ion can be prepared in a known manner. For example, a droplet of a gel solution is formed at the tip of a capillary, and a seed to be encapsulated is introduced into the droplet by making use of the capillary. The gel droplet containing a seed is dropped in a solution containing a metal ion capable of water-insolubilizing the gel. Such a metal ion is hereinafter referred to as a metal ion for coagulation, and the solution containing the metal ion which is to be used for insolubilizing the aqueous gel in water is hereinafter referred to as a coagulating solution.

The hardness of the gel coat (breaking load) can be controlled appropriately according to the kind of the seed to be encapsulated and the planting conditions by adjusting the concentration of the metal ion for coagulation or the contact time of the aqueous gel layer and the coagulating solution.

The aqueous gel forming the gel layer around a seed includes sodium alginate and sodium polyacrylate.

It is necessary that the concentration of the metal ion in the storage solution used in the invention be lower than that of the metal ion for coagulation in a coagulating solution. If the former concentration is higher than the latter concentration, the gel coat gains hardness during storage to increase the breaking load, which inhibits the germinated bud or root from sticking out. On the other hand, if the former concentration is too much lower than the latter concentration, the strength of the gel coat is reduced during storage. Accordingly, the metal ion concentration in the storage solution should be adjusted depending on that of the solution for coagulation. A preferred metal ion concentration in the storage solution is usually 0.001 to 0.6% by weight, and still preferably 0.005 to 0.06% by weight for obtaining higher effects.

The metal ions for coagulation which can be used in the invention include divalent metal ions, such as a calcium ion and a barium ion, and an aluminum ion. These metal ions are usually added to the storage solution in the form of a chloride.

It is required that the storage solution has such an osmotic pressure that gives substantially no adverse influence on the compressive breaking strength of the gel coat. The term "substantially" as used here is explained below. The gel coat of a gel-coated seed which has been adjusted to have a hardness appropriate for the seed sometimes undergoes change in compressive breaking strength when immersed in a storage solution. This being the case, as far as the compressive breaking strength after the storage is within 70 to 130% of the initial compressive breaking strength, the osmotic pressure of the storage solution is regarded to have an osmotic pressure that gives substantially no adverse influence on the compressive breaking strength of the gel coat.

When gel-coated seeds are stored in a storage solution having such an osmotic pressure, the gel-coated seeds undergo substantially no change in properties except compressive breaking strength, such as visibility and size of the encapsulated seed, and the surface conditions of the gel coat, as compared with the gel-coated seeds immediately after preparation. Thus, there is no mistaking in observing the germination, which may lead to an error of judgement of the proper time of planting. Because the size of the gel-coated seed is also substantially unchanged, various parts of a planting machine, such as a grating, as designed for gel-coated seeds immediately after preparation can be used in common for the gel-coated seeds after storage.

For osmotic pressure control, the storage solution may contain various compounds other than the metal ion for

coagulation. Compounds useful to this effect include nonionic substances (e.g., polyethylene glycol), sodium chloride, potassium nitrate, and ammonium sulfate. It should be noted that a choice of the compound to be added be made so as not to adversely affect the gel coat and the encapsulated seed. Of the above-mentioned compounds, potassium nitrate and ammonium sulfate are preferred, for they serve as a fertilizer component after planting to accelerate growth of the plant.

An osmotic pressure is generally obtained by van't Hoff's equation. Furthermore, the composition of a storage solution having an osmotic pressure that gives no substantial influence on the compressive breaking strength of the gel coat as mentioned above can be obtained through a preliminary experiment as follows.

Aqueous solutions having a varied osmotic pressure are prepared by mixing the above-mentioned salts in a varied ratio, and a small amount of gel-coated seeds are immersed in each solution for one week at a prescribed storage temperature. The change in breaking strength of the gel-coated seeds due to the immersion is examined, and those solutions in which the gel-coated seeds show a rate of change in breaking strength within ±30% are selected. The above-described preliminary experiment for deciding the composition of a storage solution may be carried out using gel capsules containing no seed in place of gel-coated seeds.

In the present invention, gel-coated seeds are stored at a storage temperature controlled at 0° to 10° C. Some storage solutions may not freeze at temperatures below 0° C. because of the presence of a solute, but such low temperatures tend to adversely affect germination of the seeds. Therefore, the storage temperature should be 0° C. or higher. The allowable storage period is extended at temperatures of 10° C. or lower, while varying depending on the kind of seeds. Temperatures of from 0° to 5° C. are recommended for obtaining the best results.

The present invention provides a method of storing gel-coated seeds having a gel coat comprising an aqueous gel which has been water-insolubilized with a metal ion. The present invention makes it possible to store the gel-coated seeds while preventing evaporation loss of water from the gel coat thereby maintaining a water content necessary for germination.

Since the gel-coated seeds stored according to the present invention for about 20 days can retain the characteristics of the gel coat thereof, i.e., size, surface conditions, and visibility of the encapsulated seeds, they can be handled in the same manner as for those immediately after preparation. Therefore, a series of operations of from coating with gel to planting can be carried out efficiently. The gel-coated seeds thus stored exhibit equal rate of germination and rate of sticking out to those of the gel-coated seeds immediately after preparation.

In addition, the storage method of the invention requires no expensive chemicals such as water-absorbing polymers and is therefore very economical.

The present invention will now be illustrated in greater detail with reference to Examples, but it should be understood that the invention is not deemed to be limited thereto. Unless otherwise indicated, all the percents are by weight.

EXAMPLES

Decision of Composition of Storage Solution

Droplets of a 3% aqueous solution of sodium alginate were dropped in a 10% aqueous solution of calcium chloride to prepare 100 water-insoluble gel capsules having a diameter of 10 mm.

The breaking load of 20 gel capsules out of 100 was measured with a rheometer equipped with a 2 kgf load cell. In more detail, filter paper for non-slip was placed on the lower disc stage of the measuring part of the rheometer, and a sample gel capsule was put thereon. The lower stage was lifted to crush the sample by compression with the upper disc stage. A breaking strength was obtained from the stress-strain curve. Measurement was made for 20 gel capsules to obtain an average.

The rest of the gel capsules were immersed in a 0.08% aqueous solution of calcium chloride kept at 5° C. for 7 days. Thereafter, the breaking load of the gel capsules was measured in the same manner as described above. As a result, the average breaking load of the immersed gel capsules was found to be within ±20% of that of the gel capsules as measured immediately after preparation. The 0.08% calcium chloride aqueous solution was thus proved suitable as a storage solution to be used in Example.

Example 1 and Comparative Examples 1 and 2

A droplet of a 3% sodium alginate aqueous solution was formed at the tip of a capillary, and a radish seed was introduced into the droplet by making use of the hollow part of the capillary. Each droplet containing one seed was dropped in a 10% aqueous solution of calcium chloride to prepare 2500 water-insolubilized gel-coated seeds having a diameter of 10 mm (hereinafter designated gel-coated seeds A).

Measurement of the breaking load of 20 gel-coated seeds A out of 2500 gave an average of 0.5 kgf. A hundred gel-coated seeds A weighed 88 g in average.

In a 0.08% calcium chloride aqueous solution were immersed 700 gel-coated seeds A and refrigerated at 2° C. (Example 1).

For comparison, 700 gel-coated seeds A were refrigerated as such at 2° C. and 55% RH (Comparative Example 1), and 1000 gel-coated seeds A were stored as such in a thermo-hygrostat at 20° C. and 65% RH.

The breaking load and the weight (for 100) of these gel-coated seeds after 3, 7, 10, and 20 days storage. The weight of gel-coated seeds is a measure of change in water content of the gel coat.

Furthermore, 100 gel-coated seeds A each of Example 1 and Comparative Examples 1 and 2 after 20 days' storage were planted on a dish having a diameter of 12 cm and kept in the dark at 20° C. The rate of germination and rate of sticking out (bud's or root's sticking out through the gel coat) were examined everyday for consecutive 7 days.

The results obtained are shown in Tables 1 to 4 below.

TABLE 1

	Change of Breaking Load (kgf)		
Time of Storage	Example 1	Compara. Example 1	Compara. Example 2
Immediately after preparation	0.58	0.57	0.56
1 day	—	0.71	0.86
2 days	—	0.92	unmeasurable*
3 days	0.60	1.51	"
4 days	—	unmeasurable*	"

TABLE 1-continued

Change of Breaking Load (kgf)

Time of Storage	Example 1	Compara. Example 1	Compara. Example 2
7 days	0.59	"	"
10 days	0.61	"	"
20 days	0.60	"	"

Note: *The breaking load was too high to be measured with a load cell of 2 kgf.

TABLE 2

Weight of 100 Gel-Coated Seeds (g)

Time of Storage	Example 1	Compara. Example 1	Compara. Example 2
Immediately after preparation	87.4	87.4	87.5
1 day	—	70.2	60.9
2 days	—	63.2	43.6
3 days	87.5	55.1	26.6
4 days	—	18.2	13.6
7 days	87.2	5.9	3.7
10 days	87.1	4.2	3.7
20 days	87.3	3.7	3.7

TABLE 3

Rate of Germination (%)

Time of Storage	Example 1	Compara. Example 1	Compara. Example 2
Immediately after preparation	3	3	21
1 day	14	3	21
2 days	92	3	18
3 days	98	2	18
4 days	98	2	18
5 days	98	2	18
6 days	98	2	18
7 days	98	2	18

TABLE 4

Rate of Sticking out (%)

Time of Storage	Example 1	Compara. Example 1	Compara. Example 2
Immediately after preparation	0	0	0
1 day	4	0	0

TABLE 4-continued

Rate of Sticking out (%)

Time of Storage	Example 1	Compara. Example 1	Compara. Example 2
2 days	56	0	0
3 days	83	0	0
4 days	95	0	0
5 days	96	0	0
6 days	96	0	0
7 days	97	0	0

The results in Tables 1 to 4 show that the gel-coated seeds stored by the method of the invention do not lose the water content in their gel coat, so that the rate of germination of the encapsulated seeds is not reduced. Since the breaking strength of the gel coat does not increase during the storage, the rate of sticking out is not reduced.

Any of the gel-coated seeds of Example 1 which had been stored for 3, 7, 10 or 20 days showed neither change of the surface conditions, such as peeling or cracks, nor change of size. The stored gel-coated seeds were utterly equal to those immediately after preparation in visibility of the encapsulated seeds.

While the invention has been described in detail and with reference to specific examples thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the spirit and scope thereof.

What is claimed is:

1. A method for storing gel-coated seeds having a gel coat comprising an aqueous gel having been water-insolubilized by a metal ion in a coagulating solution, which comprises storing the gel-coated seeds in an aqueous solution containing said metal ion, said aqueous solution containing said ion at a lower concentration than in said coagulation solution, wherein the aqueous solution has an osmotic pressure that provides substantially no influence on compressive breaking strength of the gel coat, and the gel-coated seeds are stored at a temperature of from 0° to 10° C.

2. The method as claimed in claim 1, wherein the aqueous gel comprises sodium alginate or sodium polyacrylate.

3. The method as claimed in claim 1, wherein the concentration of the metal ion in the aqueous solution is from 0.001 to 0.6% by weight.

4. The method as claimed in claim 1, wherein the metal ion is selected from the group consisting of a calcium ion, a barium ion, and an aluminum ion.

5. The method as claimed in claim 1, wherein the aqueous solution further contains polyethylene glycol, sodium chloride, potassium nitrate, or ammonium sulfate.

* * * * *